

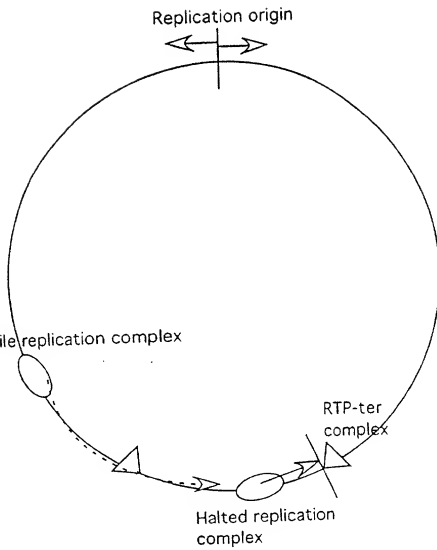
Replication origin

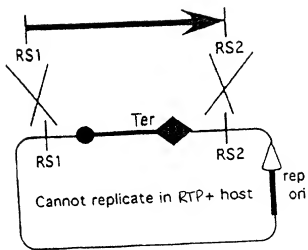
Mobile replication complex

RTP-ter  
complex

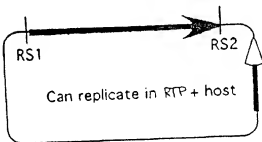
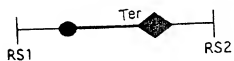
Halted replication  
complex

Fig. 1





Recombination  
reaction



Transform  
RTP+ host

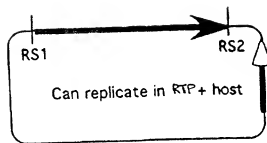
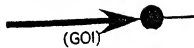
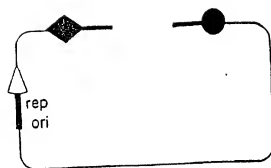


Fig. 2

Modify GOI



+



ligate

Transform RTP+ host

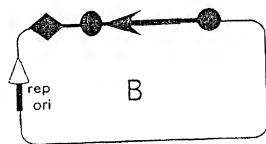
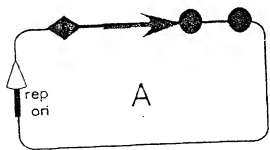
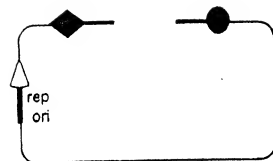


Fig. 3

gene of interest  
(GOI)

+



ligate

Transform R<sup>+</sup> host

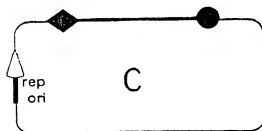
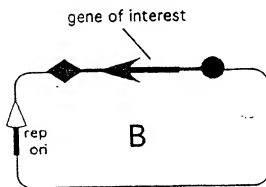
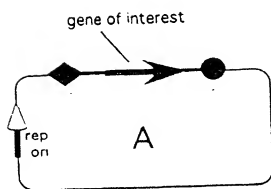


Fig. 4

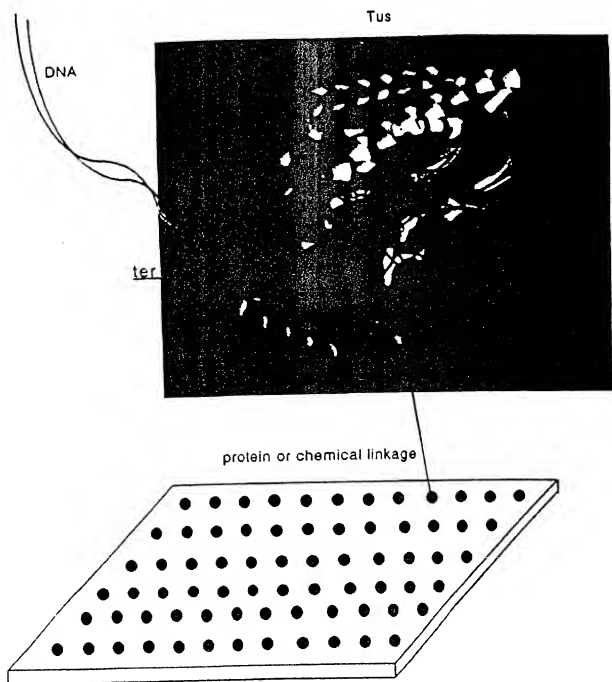
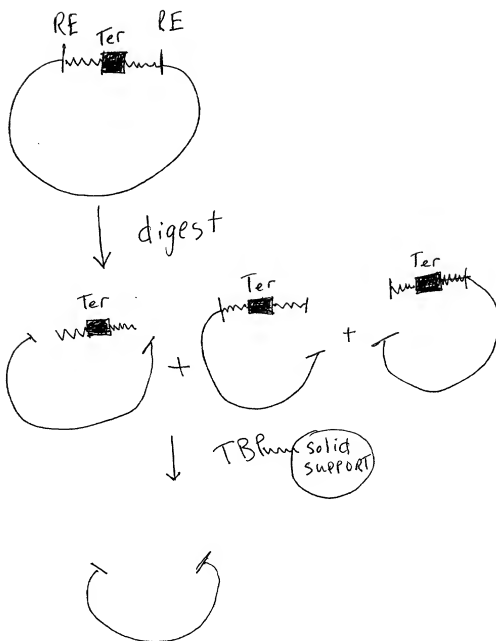


Fig. 5

FIG. 6A



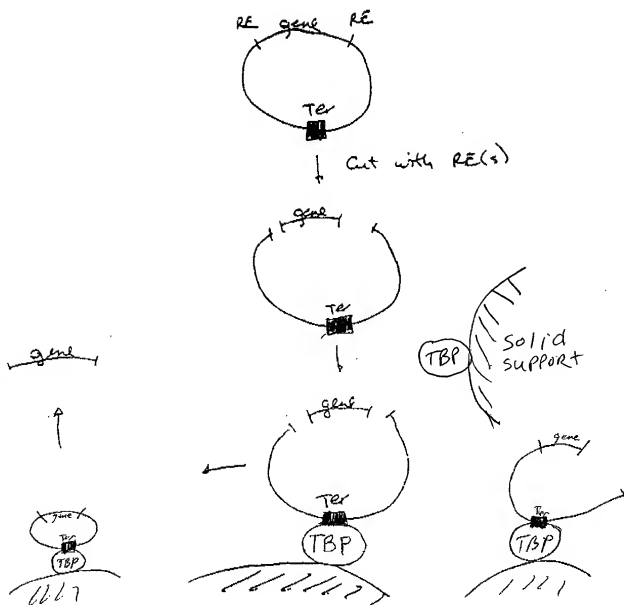


Fig. 6B

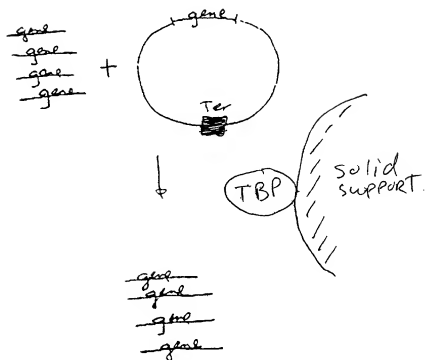
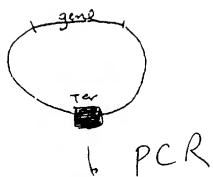


Fig. 7



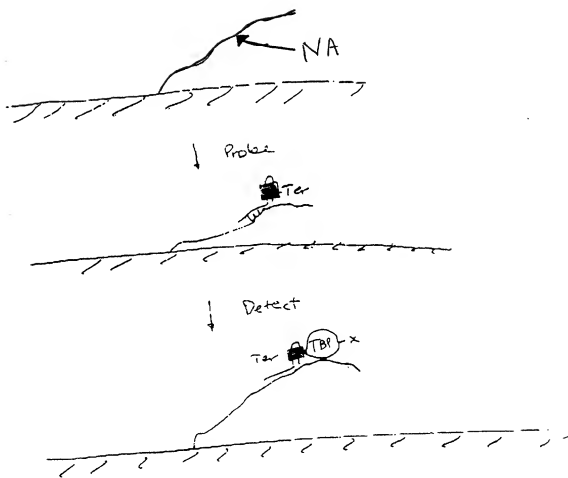
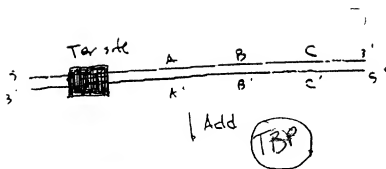


Fig. 8



↓ Add exonuclease, e.g. 3' exo

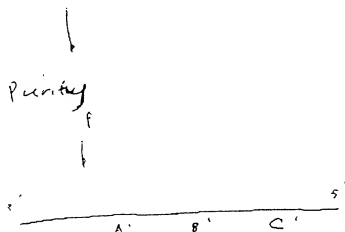
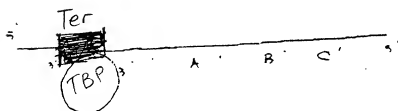


FIG 9

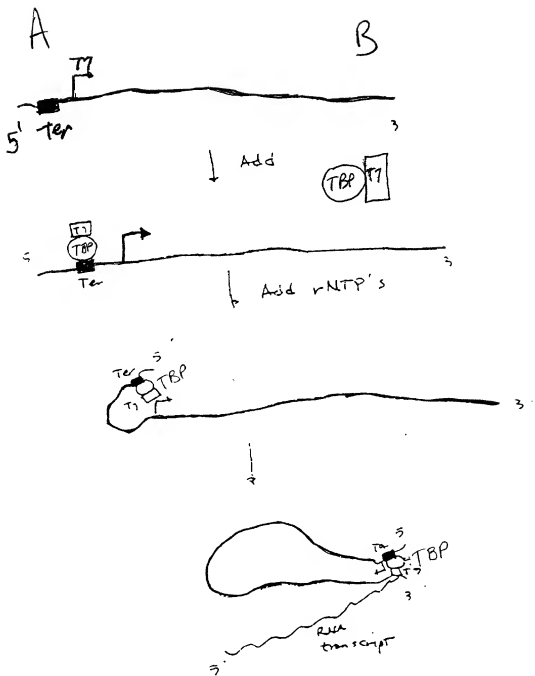


FIG 10

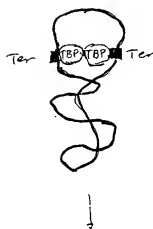
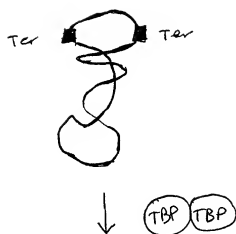


Fig. 11

A — ter' — anneal  
oligo #1

ter  
oligo #2

anneal  
A — ter' — anneal  
ter

add  
TBP

A — (ter') — anneal  
TBP

PCR  
nick

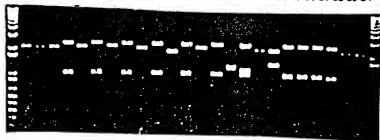
A — ( ) ————— "

deproteinize  
and remove ter

A — —————  
generate overhang

FIGURE 12

.. + + + + + - + + \* + + + +  
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24



A

.. . . . . O . O . O . . . . .  
M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24



B

Fig. 13



Fig. 14

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